Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

NATURE OF PLAGUE PROTEOTOXINS *

FREDERICK EBERSON
From the North Manchurian Plague Prevention Service, Harbin, China

In an earlier communication,[1] it was shown that shock-producing poisons could not be obtained with B. pestis when the organisms were unsensitized, however the proportions of bacteria and complement and the time element were varied. The experiments here reported are an extension of the previous studies and take up the phases of the problem of plague immunity that concern the production of powerful poisons by means of sensitized plague bacteria and the thermostability of plague proteotoxins.

Exper. 1.—One agar slant of B. pestis (24 hours) was taken up with 0.5 c.c. of NaCl solution and incubated at 37 C. for 1 hour with 0.7 c.c. of inactivated serum from Rabbit X which had been injected 5 times intravenously with B. pestis heated to 60 C. for 30 minutes. This animal received doses of 0.1, 0.2, 0.3, 0.5, and 1.0 agar slant and was bled 10 days after the last injection. The serum-bacteria mixture was centrifugated, the organisms washed once with NaCl solution and then incubated with 10 c.c. of fresh guinea-pig serum for 14½ hours at 37 C. After 1 hour's centrifugation, the supernatant fluid was injected intravenously into guinea-pigs.

TABLE 1
RESULTS OF EXPERIMENT 1

Guinea-Pig	Weight gm.	Dose c.c.	Result
1	180	1.0	No shock; died of acute plague after 72 hr.
2	266	1.5	Very slight shock? Died of acute plague after 50 hr.
3	260	4.0	Slight shock? Died of acute plague after 48 hr.

In this experiment the results were not clear-cut and it seemed as if deficient sensitization was responsible for the failure to elicit typical shock. This supposition is in all likelihood borne out by the next experiment. Here, as in the work reported in a previous paper, the few organisms remaining in the supernatant fluid were able to cause death.

---

* Received for publication January 18, 1917.

[1] Jour. Infect. Dis., 1917, 20, p. 180.

Exper. 2.—The method of procedure was the same as that followed in Exper. 1, with the exception that 2.0 c.c. of the sensitizer were used for 1½ agar slants of a Dairen strain of B. pestis and 1.9 c.c. of the same sensitizer for 1 slant of a Shanghai strain. The amount of complement used for each culture was 7.0 c.c. and the incubation period was 14½ hours.

TABLE 2

RESULTS OF EXPERIMENT 2

Guinea-Pig	Weight gm.	Dose c.c.	Result
1	172	3.5	Severe shock; typical death in 5 min.
2	150	2.5	Severe shock; died within 20 hr.
3	144	3.25	Slight shock; died within 20 hr.
4	148	3.0	Moderate shock; died within 20 hr.

Definite shock could be produced with B. pestis, with the sensitizer used, when the organisms were homologous as well as heterologous. In the case of Guinea-pigs 3 and 4, the Shanghai strain was sensitized with its homologous serum, and in 1 and 2, a Dairen strain was used with the rabbit serum which was obtained originally by injections of a Shanghai culture.

Exper. 3.—In this experiment the proteotoxins were heated for 30 minutes at 50 C.

Three agar slants of B. pestis were taken up with 0.8 c.c. NaCl solution and incubated with 3.5 c.c. of inactivated serum from Rabbit X for 1 hour at 37 C. Fifteen c.c. of guinea-pig serum were incubated with the washed, sensitized bacteria for 14 hours. Injections were made intravenously with the supernatant fluid obtained after 1 hour's centrifugation.

TABLE 3

RESULTS OF EXPERIMENT 3

Guinea-Pig	Weight gm.	Dose c.c.	Result
1	168	4.0	Severe shock; died in 6 hr.
2	150	3.5	Severe shock; died of acute plague after 48 hr.
3	142	3.0	Severe shock; died in 18 hr.
4	151	3.5	Severe shock; died in 21 hr.

Heating at 50 C. for one-half hour does not destroy the shock-producing properties of proteotoxin obtained with B. pestis. Guinea-pigs 1 and 2 served as controls, unheated material being used for injection.

Exper. 4.—In this and the following experiments, rabbits were substituted for guinea-pigs and horse serum was used instead of guinea-pig serum for the production of proteotoxins.

One and one-half agar slants of B. pestis were sensitized at 37 C. for 1 hour with 1.8 c.c. inactivated serum from Rabbit X. Ten c.c. of normal horse serum were incubated for 14 hours in contact with the washed, sensitized bacteria. After centrifugation, the supernatant fluid was heated for 35 minutes at 50-51 C. and 5 c.c. were injected intravenously in a rabbit weighing 567 grams. The rabbit had a severe shock and fell on its side at once. Dyspnea, twitching and pawing occurred. It recovered in 1 minute. The next day the animal died. Three agar slants of B. pestis were incubated for 1 hour at 29 C. with 4.0 c.c. of inactivated serum from Rabbit X. Twenty c.c. of normal horse serum were incubated with the sensitized organisms for 16 hours at 26-29 C. The supernatant fluid was heated for 30 minutes at 51-52 C.

TABLE 4

RESULTS OF EXPERIMENT 4

Rabbit	Weight gm.	Dose c.c.	Result
2	412	2.5	Moderate shock; fell to side; dyspnea, pawing and trembling; recovered in 1 min.
3	297	3.0	Slight shock, recovered in 1 min.

One and one-half agar slants B. pestis were used with 2.0 c.c. of the sensitizer from Rabbit X and incubated for 1 hour at 30 C. Ten c.c. of normal horse serum were incubated for 14 hours at 30-31 C. The proteotoxin was heated for 30 minutes at 60-61 C. and 2 c.c. were injected into a rabbit weighing 345 gm. Severe shock resulted. It fell on its side at once. Dyspnea and continuous pawing occurred. The animal recovered in 2 minutes.

The preceding experiments demonstrate clearly that the proteotoxins obtained by the method given are thermostabile. Heating for 30-35 minutes at temperatures ranging from 50-61 C. does not destroy the toxicity or shock-producing properties.

There is a wide range of combinations within which the poisons may be obtained, as is evident from the various conditions of temperature and incubation.

Exper. 5.—In this experiment, heavier animals were used and the results of the previous trials were confirmed. Four slants of B. pestis were emulsified in a total volume of 1.0 c.c. NaCl solution and incubated with 5.5 c.c. of serum (inactivated) from Rabbit X, for 1 hour at 29 C. Thirty c.c. of normal horse serum were used for the complex. The incubation period was 14 hours at 29 C. The proteotoxin was divided into 2 lots and heated at 50 C. for 35 minutes and at 57 C. for 30 minutes, respectively. The injections, as before, were made into the marginal ear vein.

The potency of the poison is well shown in this experiment, in which an effort was made to administer doses sufficiently great to induce shock, yet enabling the animals to survive. Unless the dose given was well below that required for visible shock, according to the weight of the animal, the outcome was invariably fatal. Death seemed to be due to a slow poisoning. This has been observed in numerous other instances which have been omitted in this report for the sake of brevity.

TABLE 5

RESULTS OF EXPERIMENT 5; INJECTION OF PROTEOTOXIN HEATED AT 57 C

Rabbit	Weight gm.	Dose c.c.	Result
1	726	2.4	No shock.
2	776	3.2	Moderate shock; on side immediately; intermittent pawing and twitching; recovery in 2 min.; died in 20 hr.
3	587	4.0	Very severe shock; dyspnea, continuous pawing and trembling; recovered in 7 min.; died after 6 days with severe emaciation.

An analysis of these experiments indicates that the toxemic manifestations of plague bacteria are of a different nature from those which have been described for other organisms such as the typhoid bacillus, for example. The most striking feature of B. pestis from the standpoint of poison-production is the strict requirement of sensitization. In the case of other bacteria studied, this has never been encountered, so far as I know. Sensitization has usually hastened the formation of proteotoxins, but has not been an indispensable factor in the process, since unsensitized bacteria gave rise to powerful poisons equally well. The significance of this point does not immediately become apparent, but suggests possible explanations for the mechanism of plague infection, the relationship existing between proteotoxins and bacterial virulence and the possible importance of an antigen-sensitizer complex in the production of shock.

TABLE 6

RESULTS OF EXPERIMENT 5: INJECTION OF PROTEOTOXIN HEATED AT 50 C.

Rabbit	Weight gm.	Dose c.c.	Result
1	973	2.0	No shock
2	883	3.6	Very slight shock; died after 6 days
3	680	2.5	Slight shock; died after 6 hr.

Much evidence has accumulated to support the belief that the different heat-stable antibodies are fundamentally alike. From the results recorded in the preceding experiments, it seems as if we might safely identify such heat-stable poisons with one of these antibodies. Zinsser[2] has shown conclusively that after stated intervals, resistance may be developed to shock, subsequent to a single injection of proteotoxins obtained with typhoid bacilli. If thermostability can be proved for these proteotoxins, it will mean the formulation of a general principle.

The rôle played by the sensitizer in the mechanism of shock when B. pestis is used suggests the likelihood of an antigen-sensitizer complex as the important factor in plague infection. This becomes a point of departure for the idea that bacterial virulence and the ability to liberate proteotoxins may be correlated in some instances. In an earlier paper, it was shown that B. pestis became distinctly enhanced in virulence after being incubated with normal guinea-pig serum. At first it was thought that this phenomenon was limited to treatment with normal serum, but such is not the case, since heated serum had a like action. This has been demonstrated also by Rowland,[3] working with horse serum. The nature of this mechanism has not been defined clearly, if at all, since the function of bacteria in the production of shock has been variously explained. Recent studies by Jobling and Peterson[4] show that bacteria remove the serum-antiferments, thus liberating the ferments normally present in the serum and enabling them to act on the constituent proteins. This conception of the mechanism of shock does not explain entirely the ability to obtain proteotoxins by means of inactivated sera, as has been shown by Neufeld and Dold;[5] rather, it suggests that the thermostabile substance acts like a sensitizer and that the increased 'virulence' noted in this paper is to be explained possibly by the action of complement upon the antigen-sensitizer complex, after the bacteria have been able to multiply sufficiently by virtue of their increased resistance.

Virulence and the ability to produce proteotoxins, then, seem to be synonymous. The unusual susceptibility of the guinea-pig to plague infection may fall into line with this conception. As has been seen, B. pestis becomes more virulent after contact with serum from this animal. Some substance, in all likelihood normal sensitizer, is absorbed out of the serum and the combination so formed becomes amenable to the action of complement. The ease and rapidity with which proteotoxins may be produced will determine the degree of virulence by which the organism in question may be characterized.

---

[2] Jour. Exper. Med., 1914, 20, p. 582.
[3] Jour. Hyg., Plague Supplement, 1912.
[4] Jour. Exper. Med., 1914, 20, p. 480.
[5] Cited by Zinsser in Infection and Resistance, 1916, p. 424.

SUMMARY AND CONCLUSIONS

Powerful shock-producing poisons can be obtained by allowing normal guinea-pig or horse serum to act upon sensitized plague bacteria.

The proteotoxins of the plague bacteria are thermostabile, resisting prolonged heating at temperatures ranging from 50-61 C.

A wide range exists within which the poisons may be obtained. The essential requirement is sufficient sensitization of the plague organisms.

Proteotoxins are obtainable even after considerable exposures. The average period used in these experiments was 14 hours, exclusive of the hour or more necessary for centrifugation and the time required during subsequent operations. In several instances a period as long as 18 hours did not preclude the formation of the toxic substances.

The mechanism of plague infection seems to depend on a primary sensitization of the organisms, the antigen-sensitizer complex being important for poison-production. Virulence, so far as B. pestis is concerned, is probably an altered state of the bacteria which caused poisons to be liberated when the normal serum components are allowed to act on the newly-formed complex.